Amendments to the Specification

Please note that "strikeout" matter is shown with larger-than-normal italic letters containing the strikeout horizontal marks such as in this example: ~~*strikeout*~~.

Please currently amend the specification on page 3, TABLE 1, as follows:

~~*TABLE 1*~~

~~*Electrode Chamber Volume milliliters*~~

~~*Number Out Million /ml*~~	~~*Number In Million /ml*~~	~~*Cell Density 20 million /ml*~~	~~*40 million/ml*~~
~~*10*~~	~~*20*~~	~~*1*~~	~~*0.5*~~
~~*100*~~	~~*200*~~	~~*20*~~	~~*5*~~
~~*1000*~~	~~*2000*~~	~~*100*~~	~~*50*~~

TABLE 1			
Electrode Chamber Volume in Milliliters			
Number of Cells Out	Number of Cells In	Cell Density	
Million/ml	Million/ml	20 million/ml	40 million/ml
10	20	**1**	**0.5**
100	200	**20**	**5**
1000	2000	**100**	**50**

Please currently amend the specification, in the paragraph spanning page 3 line 25 to page 4 line 2, as follows:

Clinical application generally requires 10 million to 500 million cells in which the large molecules have been properly inserted. If a treatment requires 10 million cells per dose (treatment) and 5 doses are required, at least 50 million therapeutic cells must be prepared. If the efficiency of the electroporation process is assumed to be 50% and cells are treated at a ~~*concentration*~~ cell density of 20 million cells/ml then a 5 ml capacity ~~*electrode*~~ chamber would be required (50 million X 2 / 20 million). If 100 million therapeutic cells are required, a 10 ml capacity ~~*electrode*~~ chamber would be needed.

Please currently amend the specification, in the paragraph on page 4, spanning lines 3-9, as follows:

Simply increasing the size of the ~~*electrode*~~ chamber to

achieve the desired ~~capacity is not practical because this causes~~ a proportionate increase in amperage due to a decrease in resistance ~~*in the electrode*~~ of the suspension within the chamber. ~~As the size of the *electrode* chamber increases, the~~ resistance of the ~~*electrode*~~ suspension within the chamber decreases as long ~~as the conductivity of the *medium used*~~ suspension in the chamber remains constant.

Please currently amend the specification, in the paragraph on page 4, spanning ~~lines 10-12, as follows~~:

~~If *a 100*~~ 200 million therapeutic cells are required and the input cell density is 20 million cells per milliliter, then a 20 ml ~~*electrode*~~ chamber ~~is required~~.

Please currently amend the specification, in the paragraph on page 4, spanning ~~lines 13-19, as follows~~:

In this case just scaling the size of ~~*the electrode*~~ a chamber up to 20 ~~milliliters does not work~~. ~~As the volume of *the electrode*~~ a suspension within chamber increases, the resistance of the ~~*electrode due to the conductivity of the media*~~ suspension within the chamber ~~decreases~~. ~~The~~

resistance of the ~~*media*~~ suspension in the ~~*electrode*~~ chamber is calculated as follows:

Please currently amend the specification, in the paragraph on page 5, spanning lines 3-5, as follows:

The TABLE 2 below shows the ~~*electrode chamber resistance*~~ resistance of the suspension within the chamber as a function of volume for a ~~4-millimeter gap and *media*~~ suspension conductivity of 0.017 Siemens/cm.

Please currently amend the specification on page 5, TABLE 2, as follows:

~~*TABLE 2*~~

~~*Electrode Volume ml*~~	~~*Media Resistance Ohms*~~
~~*0.5*~~	~~*19.2*~~
~~*1*~~	~~*9.6*~~
~~*5*~~	~~*1.92*~~
~~*10*~~	~~*0.96*~~
~~*50*~~	~~*0.19*~~

TABLE 2	
Chamber Volume ml	Suspension Resistance ohms
0.5	19.2
1	9.6
5	1.92
10	0.96
50	0.19

Please currently amend the ~~specification~~, in the ~~paragraph~~ on page 5, spanning lines 14-17, as follows:

When the ~~*electrode*~~ chamber ~~volume is~~ above 1 ml, ~~the~~ resistance of the ionic solution ~~becomes impractically small;~~ significant solution heating will occur due to the high pulse current destroying the cells.

Please currently amend the specification, in the paragraph on page 5, spanning lines 18-22, ~~as follows~~:

To address this problem a flow though technique was developed. In this process the large volume of ~~*media*~~ suspension flows through a small treatment ~~chamber, and~~ the voltage ~~pulse~~ waveform is applied to the parallel plates in the chamber. The problems with this process are:

Please currently amend the specification, in the TABLE 3 spanning pages 6-7, as follows:

~~*1. TABLE 3*~~

~~*The following table summarizes the current state of the art:*~~

~~*Publication Conductivity Cations Anions Osmolarity Buffer*~~

~~*(μs/cm) High Conc. Low Conc.*~~
~~*Invention Low (50-150) None Ca, Mg Organic L-N Histidine*~~
~~*5,124,259 High K Ca, Mg Organic N*~~
~~*6,040,184 Very low None None None L-N None*~~
~~*6,338,965 Very low None None None L-N None*~~
~~*6,368,784 High K Ca, Mg Cl N Phos, HEPES*~~
~~*Djuzenova 1996 Moderate to high (800-14000) Na, K Ca Cl, Sulfate N Phos.*~~
~~*Kinosita 1977 High Na Cl Phos.*~~
~~*Dimitrov 1990 Low to Moderate Na Phos., Cl Phos.*~~
~~*Rols 1989 Low and high Na Cl Phos.*~~
~~*Pucilar 2001 Low and high Na, K (if used) Mg Cl, Sulfate N Phos*~~

1.

The following table summarizes the current state of the art:

TABLE 3						
Publication	Conductivity	Cations		Anions	Osmolarity	Buffer
	(μs/cm)	High Conc.	Low Conc.			
Invention	Low (50-150)	None	Ca, Mg	Organic	L-N	Histidine
5,124,259	High	K	Ca, Mg	Organic	N	
6,040,184	Very low	None	None	None	L-N	None
6,338,965	Very low	None	None	None	L-N	None
6,368,784	High	K	Ca, Mg	Cl	N	Phos, HEPES
Djuzenova 1996	Moderate to high (800-14000)	Na, K	Ca	Cl, Sulfate	N	Phos.
Kinosita 1977	High	Na		Cl		Phos.
Dimitrov 1990	Low to Moderate		Na	Phos., Cl		Phos.
Rols 1989	Low and high	Na		Cl		Phos.
Pucilar 2001	Low and high	Na, K (if used)	Mg	Cl, Sulfate	N	Phos

Please currently amend the specification, in TABLE 5 spanning pages 15-16, as follows:

~~*TABLE 5*~~

~~*1.1. TABLE 5*~~
~~*2.2. Electrode DimensionsStatic, no adherent cells*~~

~~*Publication*~~	~~*Conductivity (μs/cm)*~~	~~*Electrode Dimensions*~~			~~*Cuvette*~~	~~*Volume*~~	~~*Dimension*~~
		~~*Height*~~	~~*Width*~~	~~*Gap*~~			
			~~*mm*~~	~~*mm*~~	~~*mm*~~	~~*ml*~~	
~~*5,124,259*~~	~~*High (~10K)*~~	~~*2*~~	~~*87.5*~~	~~*4*~~	~~*N*~~	~~*0.7*~~	~~*0.23*~~
~~*6,040,184*~~	~~*Very low*~~				~~*Y*~~		~~*0.1-0.4*~~
~~*6,338,965*~~	~~*Very low*~~				~~*Y*~~		~~*0.1-0.4*~~

6,368,784	High (~17K)			4			0.4
Djuzenova 1996	Moderate to high (800-14000)			6	N	1.2	0.3
*Kinosita 1977	Saline and sucrose	-	5-100	2-10	N, cross section= 50-200 mm^2	...	Not determinable from publication
Reimann 1975	PBS	30	30	10	-	...	0.11
Dimitrov 1990	Low to Moderate (~100-10K)			2	N	.003	66
Pucilar 2001	0.0011 - 1.61 S/m			2		.05	0.8
Baron 2000	High (~17K)				4	.4	0.4
Schwister 1985	PBS	30	30	10	N	10	0.11
Mussauer 2001	1.5-3.5 mS/cm				2	.4	0.1
Mussauer 1999	1-8 mS/cm			6		1.1	0.33
Fomekong 1998	0.064-1.447 S/cm			5	...	.884	0.28

~~5,128,257 Saline 10-20 50-80 0.5-1.5~~
~~5,186,800 Water 0.5-2.5 0.00-1 1 0.5-hundreds~~

TABLE 5

Electrode Dimensions; Static, no adherent cells

Publication	Conductivity	Electrode Dimensions			Cuvette	Volume	Dimension
	(μs/cm)	Height	Width	Gap			
			mm	Mm	mm	ml	
5,124,259	High (~10K)	2	87.5	4	N	0.7	0.23
6,040,184	Very low				Y		0.1-0.4
6,338,965	Very low				Y		0.1-0.4
6,368,784	High (~17K)				4		0.4
Djuzenova 1996	Moderate to high (800-14000)			6	N	1.2	0.3
*Kinosita 1977	Saline and sucrose		5-100	2-10	N, cross section= 50-200 mm^2		Not determinable from publication
Reimann 1975	PBS	30	30	10			0.11
Dimitrov 1990	Low to Moderate (~100-10K)			2	N	0.003	66
Pucilar 2001	0.0011 – 1.61 S/m			2		0.05	0.8
Baron 2000	High (~17K)				4	0.4	0.4
Schwister 1985	PBS	30	30	10	N	10	0.1
Mussauer 2001	1.5-3.5 mS/cm				2	0.4	0.1
Mussauer 1999	1-8 mS/cm			6		1.1	0.33
Fomekong 1998	0.064-1.447 S/cm			5		0.884	0.28
5,128,257	Saline	10-20	50-80		0.5-1.5		
5,186,800	Water				0.5 – 2.5	0.001- 1	0.5-hundreds

Please currently amend the specification, in the paragraph spanning page 16 line 9 to page 17 line 7, as follows:

Having discussed prior art above, it is clear that the foregoing body of prior art does not teach or suggest electroporation methods and apparatus which have the following combination of desirable features: (1) can be used for clinical and therapeutic purposes wherein all cells, ex vivo or in vitro, are subject to substantially the same process conditions; (2) is scalable so that substantially large volumes of ex vivo or in vitro cells can be processed in a relatively short period of time; (3) achieves increased biological cell capacity without increasing the size of ~~*electrodes*~~ chamber resulting in excessively large amperage requirements; (4) limits heating within the ~~*treatment cell*~~ chamber to low levels; (5) exposes substantially all ex vivo or in vitro cells to the same electric field intensity and direction; ~~*(6) provides that the density of the material to be inserted into the treatment chamber can be held constant;*~~ (~~7~~ 6) permits variable rectangular pulse waveforms such as disclosed in U. S. Patent No. 6,010,613 can be employed; (~~8~~ 7) avoids problems in flow through treatment cells that are due to laminar and turbulent flow conditions; (~~9~~ 8) permits the use of medium with lower conductivity to achieve the movement of macromolecules into mammalian cells and to allow the use of larger ~~*capacity electrodes*~~ volume chambers; and (~~10~~ 9) is easily scalable to large capacity without using a flow through treatment chamber for cells to be treated.

Please currently amend the specification, in the paragraph on page 17, spanning lines 29-33, as follows:

Yet another object of the present invention is to provide a large volume ex vivo electroporation method which achieves increased biological cell capacity without increasing the size of ~~electrodes~~ the chamber resulting in excessively large amperage requirements.

Please currently amend the specification, by deleting the paragraph on page 18, spanning lines 5-9, as follows:

~~Yet another object of the present invention is to provide a large volume ex vivo electroporation method that provides that the density of the material to be inserted into the treatment chamber can be held constant.~~

Please currently amend the specification, in the paragraph on page 18, spanning lines 19-24, as follows:

Still a further object of the present invention is to provide a large volume ex vivo electroporation method that permits the use of medium with lower conductivity to achieve the movement of macromolecules into mammalian cells and to allow the use of larger capacity ~~electrodes~~ chambers.

Please amend the specification, in the paragraph on page 19, spanning lines 3-15, as follows:

To achieve the foregoing and other advantages, the present

invention, briefly described, provides a static chamber with large volume to insure all cell are subject to the same electric field intensity and direction and the density of the cells and material are uniform. With this invention any waveform may be used. This invention is a voltage waveform generator connected to an electrode with parallel plates with low conductivity media, between the plates having 10 million cells or more. The invention uses ~~*media*~~ a suspension with conductivity between 1 microSiemens/cm and 100 milliSiemens/cm as shown in FIG. 2. The invention may be used in clinical applications and has a closed sterile chamber into which the cells and large molecules are inserted and removed.

Please currently amend the specification, in the paragraph on page 19, spanning lines 20-30, as follows:

a. retaining a suspension of the vesicles and the exogenous material in a treatment volume in a chamber which includes electrodes, wherein the chamber has a geometric factor (cm.sup.-1) defined by the quotient of the electrode gap squared (cm.sup.2) divided by the chamber volume (cm.sup.3), wherein the geometric factor is less than ~~*or equal to*~~ 0.1 cm.sup.-1) and greater than 0.000001 (cm^{-1}) , wherein the suspension of the vesicles and the exogenous material is in a medium which is adjusted such that the ~~*medium*~~ suspension has conductivity in a range spanning greater than 0.001 milliSiemens/cm to less than 100 milliSiemens/cm as shown in FIG.2, wherein the suspension is

enclosed in the chamber during treatment, and

Please currently amend the specification by deleting the paragraph on page 21, lines 3-4, as follows:

~~*With the method of the invention, the temperature rise during vesicle treatment is miniscule.*~~

Please amend the specification, in the paragraph spanning page 21, line 31 to page 22, line 6, as follows:

In accordance with another aspect of the invention, an electroporation apparatus is provided which includes a chamber which has a chamber volume of at least 2 milliliters. A pair of electroporation electrodes are contained within the chamber. An electroporation medium, carrying vesicles in suspension, is contained in the chamber between the electroporation electrodes. The ~~*medium*~~ suspension has a conductivity between 1 microS/cm and 100 milliS/cm as shown in FIG. 2. A source of pulsed voltages is electrically connected to the electroporation electrodes, and means for adding material to the chamber for electroporation treatment therein. Also, means are provided for removing treated material from the chamber.

Please currently amend the specification, in the paragraph on page 23, spanning lines 9-13, as follows:

FIG. 2 is a graph illustrating the operating range of the method of the invention, inside the triangle, and how the operating range of the invention is outside operating ranges of prior art electroporation methods, indicated by small blocks outside the triangle. Clearly, FIG. 2 shows one side of the triangle to be a herein-defined Geometric Factor, one side of the triangle to be conductivity, and one side of the triangle to be resistance in ohms. More specifically, inside the triangle, the conductivity is in a range spanning greater than 0.001 to less than 100 milliSiemens/cm; the herein-defined Geometric Factor ranges from greater than 0.000001 cm^{-1} to less than ~~or equal to~~ 0.100000 cm^{-1}; and the resistance is greater than one ohm.

Please currently amend the specification, in the paragraph spanning from page 23, line 27 to page 24, line 8 as follows:

As previously described a significant problem is the conductivity of the media ~~use~~ used in electroporation. In ~~this process~~ the process of the invention, a low conductivity ~~media~~ medium is employed to keep the total resistance of the ~~media small and virtually eliminates heating~~ suspension greater than one ohm, wherein heating in the chamber is limited to low

levels. Not just any ~~media~~ medium conductivity can be used. As the ionic content of the ~~media~~ medium is ~~reduce~~ reduced, the number of free ions that are available to build charge (voltage) across the cell member is decreased. The effect is to increase the amount of time it takes to charge the membrane. This process is described by the equation in Electroporation and Electrofusion in Cell Biology, edited by Eberhard Neumann, Arthur Sowers, and Carol Jordan, Plenum Press, 1989, on page 71. Assuming a typical cell diameter of 10 microns, the charging time is 20 microseconds at 80 µS/cm. Below 80 µS/cm the charging time become too long and the pathways in cell membrane stop forming. The TABLE 6 below illustrates the resistance of the media as a function of electrode chamber volume and conductivity.

Please currently amend the specification on page 24, TABLE 6, as follows:

~~TABLE 6~~

~~Electrode Volume ml~~	~~Media Resistance -- ohms~~		
	~~17,000 µS/cm~~	~~200 µS/cm~~	~~80 µS/cm~~

~~0.5 19.2 1600 4000~~
~~1 9.6 800 2000~~
~~5 1.92 160 400~~
~~10 0.96 80 200~~
~~50 0.19 16 40~~

TABLE 6			
Chamber Volume ml	Suspension Resistance - ohms		
	17,000 μS/cm	200 μS/cm	80 μS/cm
0.5	19.2	1600	4000
1	9.6	800	2000
5	1.92	160	400
10	0.96	80	200
50	0.19	16	40

Please currently amend the specification, in the paragraph on page 24, spanning lines 16-29, as follows:

Ex vivo electroporation has been demonstrated in numerous published research projects. At this point commercial applications, such as clinical transfection to produce a vaccine for the patient, requires large electrodes or chambers to process millions of cells at one time. The static parallel plate chamber provides the most uniform amplitude and most uniform electric field direction of any configuration available. This uniformity is required to insure uniform treatment of the target cells. ~~*It is also important not to use very high density cell*~~

~~*concentration such as 30 million cells/ml to insure local uniform electric fields about the cells.*~~ This invention applies to chambers larger than ~~1~~ 2 milliliter.

Please currently amend the specification, on page 25, spanning lines 10-20, as follows:

~~*ρ = resistivity in ohm-cm*~~

~~*σ = 1/ρ in Siemens/cm*~~

~~*υ = volume of material being treated*~~

ρ = resistivity in ohm-cm

σ = 1/ρ in Siemens/cm

ν = volume of material being treated (ml)

l = gap between electrodes (cm)

A = area of electrode (cm^2)

Please currently amend the specification, in the paragraph on page 25, spanning lines 33-38, as follows:

Another aspect of the invention further increases capacity by alternately filling and emptying the gap between the

electrodes. In this manner, all desired properties are met during a specific treatment and the electrodes can be re-used for subsequent treatments in ~~*an intermittent*~~ a sequential batch process.

Please amend the specification, in the paragraph spanning page 25, line 39 to page 26, line 13, as follows:

This present invention specifies a range of ~~*material*~~ suspension conductivities, which can be used versus the chamber dimensions, the larger the volume the smaller the conductivity. This invention specifies an operating area for use with the larger volume ~~*electrodes*~~ chambers. This is illustrated in FIG. 2. Operating points of prior art published results are also presented in FIG. 2 as squares. For chambers with a Geometric Factor less than 0.1 there are two limiting factors, which are related. The first is the absolute value of the chamber resistance. In this invention the chamber resistance is one ohm or greater. Operating below one ohm is viewed as impractical. The other constraint is the conductivity of the ~~*medium*~~ suspension in the chamber. As the conductivity decreases the charging time of the cell membrane increases because there are fewer ions external to the cell membrane. More specifically with respect to FIG. 2, an "Operating Region of the Invention" is

clearly shown to be a triangular region. The topmost point of the triangular "Operating Region of the Invention" has the coordinates of (along the horizontal axis) Geometric Factor in cm^{-1} of 0.100000 and (along the vertical axis) Conductivity in microSiemens/cm of 100,000.00. In addition, the far right bottommost point of the triangular "Operating Region of the Invention" has the coordinates of (along the horizontal axis) Geometric Factor in cm^{-1} of 0.100000 and (along the vertical axis) Conductivity in microSiemens/cm of 1.00. In addition, for the triangular "Operating Region of the Invention", the resistance R equals 1 ohm. It is noted that 100,000 microSiemens/cm equals 100 milliSiemens/cm. It is also noted that 1.00 microSiemens/cm equals 0.001 milliSiemens/cm.

Please currently amend the specification, in the paragraph on page 27, spanning lines 14-22, as follows:

The preferred operating region of the present invention is then:

Cell diameter > 1 micrometer

Chamber volume > 2 milliliters

Conductivity of Material to be treated > 1 microSiemens/cm

Conductivity of Material to be treated < 100,000 microSiemens/cm

Total resistance of material to be

treated in chamber	> 1 ohm
Geometric Factor of Chamber	< 0.1 cm-1

Please amend the specification, in the paragraph on page 27, spanning lines 24-31, as follows:

The invention uses a static chamber with large volume to insure that all cells in suspension are subject to the same electric field intensity and direction and the density of the cells and treating material are uniform. With this invention any waveform may be used. This invention is a voltage waveform generator connected to ~~an electrode~~ electrodes with parallel plates with ~~has~~ low conductivity ~~medium~~ suspension, having 10 million cells or more.

Please amend the specification, in the paragraph on page 27, spanning lines 32-37, as follows:

A component of the invention is the use of low conductivity medium within a defined range to limit amperage and heat while simultaneously providing enough ions to effectively electroporate cells. Typically the ~~medium~~ suspension used will have a conductivity between 10 microS/cm and 100 milliS/cm.

Please currently amend the specification, in the paragraph on page 28, spanning lines 4-9, as follows:

One aspect of the invention further increases capacity by alternately filling and emptying the ~~*electrode*~~ chamber. In this manner, all desired properties are met during a specific treatment and the electrode can be re-used for subsequent treatments in ~~*an intermittent*~~ a sequential batch process.

Please currently amend the specification, in the paragraph on page 28, spanning lines 10-24, as follows:

The conductivity of the medium used in electroporation is an important aspect of this invention. In this process, a low conductivity medium is employed to keep the total resistance of the ~~*medium small and virtually eliminate heating*~~ suspension greater than one ohm, and heating in the chamber is limited to low levels. There is a limit to the lower conductivity medium that can be used. As the ionic content of the medium is reduced the number of free ions that are available to build charge (voltage) across the cell membrane is decreased. The effect is to increase the amount of time it takes to charge the membrane. This process is described by the equation in Neumann, p71. Assuming a typical cell diameter of 10 microns, the charging time is 20 microseconds at 80 microS/cm. Below 80

microS/cm the charging time becomes too long and the pathways in cell membranes stop forming.

Please currently amend the specification, in the paragraph spanning from page 29 line 18, to page 30 line 19, as follows:

It is apparent from the above that the present invention accomplishes all of the objects set forth by providing a large volume ex vivo electroporation method which may advantageously be used for clinical and therapeutic purposes wherein all cells, ex vivo or in vitro, are subject to substantially the same process conditions. With the invention, a large volume ex vivo electroporation method is provided which is scalable so that substantially large volumes of ex vivo or in vitro cells can be processed in a relatively short period of time. With the invention, a large volume ex vivo electroporation method is provided which achieves increased biological cell capacity without increasing the size of ~~*electrodes*~~ a chamber resulting in excessively large amperage requirements. With the invention, a large volume ex vivo electroporation method is provided which limits heating within the ~~*treatment cell*~~ chamber to low levels. With the invention, a large volume ex vivo electroporation method is provided which exposes substantially

all ex vivo or in vitro cells to the same electric field intensity and direction. With the invention, a large volume ex vivo electroporation method provides that the density of the material to be inserted into the treatment chamber can be held constant. With the invention, a large volume ex vivo electroporation method is provided which permits variable rectangular pulse waveforms such as disclosed in U. S. Patent No. 6,010,613 can be employed. With the invention, a large volume ex vivo electroporation method is provided which avoids problems in flow through treatment cells that are due to laminar and turbulent flow conditions. With the invention, a large volume ex vivo electroporation method is provided which permits the use of medium with lower conductivity to achieve the movement of macromolecules into mammalian cells and to allow the use of larger capacity ~~*electrodes*~~ chambers. With the invention, a large volume ex vivo electroporation method is provided which is easily scalable to large capacity without using a flow through treatment chamber for cells to be treated.